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Dr J. Greagor Sutcliffe

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The Scripps Research Institute
10666 North Torrey Pines Road
La Jollia CA 92037

8. PERFORMING ORGANIZATION REPORT NUMBER

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AFOSR/NL
110 Duncan Ave Suite B115
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The suprachiasmatic nucleus (SCN) of the hypothalamus is the anatomical seat of the mammalian endogenous biological clock which regulates the temporal expression of hormonal and behavioral circadian rhythms. Light, serotonin and melatonin are the dominant stimuli that affect the phase of the endogenous clock. The grantee has devised strategies to identify molecules that mediate the action of these stimuli within the sCN. The grantee has identified a novel receptor for serotonin, the 5-HT7 receptor, and determined its aminoacid structure. Its pharmacological ligand binding properties ahve been measured and a unique profile of agonists and antagonists defined. These allowed demonstration that the 5-HT7 receptor mediated circadian activity of cultured SCN. The receptor has been shown to couple to activation on adenylyl cyclase and to be synthesized by neurons of the subparaventricular zone immediately dorsal to the SCN. Molecules whose expression within the sCN is activated by light entraining cues have also been identified and their characterization is under way.

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Molecular Approach to Hypothalamic Rhythms
P.I.: J. G. Sutcliffe, Ph.D.
The Scripps Research Institute
10666 N. Torrey Pines Road
La Jolla, CA 92037

I. SUMMARY

The suprachiasmatic nucleus (SCN) of the hypothalamus is the anatomical seat of the mammalian endogenous biological clock which regulates the temporal expression of hormonal and behavioral circadian rhythms. Light, serotonin and melatonin are the dominant stimuli that affect the phase of the endogenous clock. The grantee has devised strategies to identify molecules that mediate the action of these stimuli within the SCN. The grantee has identified a novel receptor for serotonin, the 5-HT_7 receptor, and determined its amino acid structure. Its pharmacological ligand binding properties have been measured and a unique profile of agonists and antagonists defined. These allowed demonstration that the 5-HT_7 receptor mediated circadian activity of cultured SCN. The receptor has been shown to couple to activation of adenylyl cyclase and to be synthesized by neurons of the subparaventricular zone immediately dorsal to the SCN. Molecules whose expression within the SCN is activated by light entraining cues have also been identified and their characterization is under way.

II. OBJECTIVES

The goal of the supported studies was to investigate the molecular mechanism that determine circadian rhythms by isolating cDNA clones of mRNAs expressed in the rodent suprachiasmatic nucleus that encode proteins involved in generating, regulating and responding to circadian environmental cues. In particular, studies were proposed to determine the receptors mediate entrainment by indoleamine neurotransmitters and to find the mRNAs whose expression is mediated by entraining cues, or that cycle circadianly within the SCN, or which are exclusive to the SCN.

III. STATUS

A. Background

Circadian rhythms are endogenously driven oscillations with approximately 24 hour periodicity. In mammals, sleep-wake patterns, water and food intake, and wheel running are examples of circadian behaviors which free run (are cyclic) under constant environmental conditions. In addition, changes in body temperature, melatonin and corticosteroid concentrations, pineal N-acetyl transferase activity and the B_{max} of several neurotransmitter receptors are parameters which follow an

endogenous circadian rhythm. The phase of circadian rhythms is altered (entrained) by external environmental cues, such as light, diet and activity. Entrainment, therefore, allows an organism to adapt its rhythms to changes in its environment.

The isolation of mutant strains of hamsters and mice that have altered circadian periodicity demonstrated that aspects of circadian rhythms are genetically determined. Anatomical ablation studies demonstrated that the seat of these endogenously generated rhythms within the brain was the ventromedial region of the hypothalamus. Subsequently, specific lesions of the suprachiasmatic nucleus (SCN) were found to disrupt normal circadian rhythmicity. Rhythms could be restored to ablated animals by SCN transplants. Final proof that the SCN contains a circadian pacemaker was established in transplantation studies using normal hamsters and the mutant strain with altered circadian period: the period of overt behavioral rhythmicity in ablated recipients was governed by the genotype of the donor transplanted SCN. The anatomical and genetic data in combination suggested that one or several clock gene products are responsible for generating circadian rhythms within the SCN. Previously, clock genes had been isolated only from invertebrate and non-metazoan species: mutations in the <u>per</u> and <u>frq</u> loci disrupt normal periodicity in Drosophila and Neurospora respectively.

There are three conceptually distinguishable components to the circadian clock: environmental cue entrainment, the clock itself, and the systems affected by the circadian clock. Several groups have shown that the expression of immediate early genes (IEGs) such as c-fos in the SCN is highly correlated with light entrainment of the circadian rhythm in hamsters and rats. Since the IEGs primarily encode transcriptional factors, these observations suggested that the entrainment pathway within SCN neurons works through a hierarchy of transcriptional events that couple the clock mechanism to physiological outputs. Thus, induction of IEGs in the SCN by light may be the first in a cascade of transcriptional events that are necessary for clock entrainment to occur. In other neuronal systems, rapid induction of IEG mRNA expression occurs in response to neurotransmitters, growth factors and membrane electrical activity. The IEGs serve as nonspecific molecular conduits between short-term events and long-term cellular changes (proliferation, differentiation, sensitization). The specificity for the long-term events must be governed by the activation or repression of a predetermined subset of the target genes of IEGs. The identification of the IEG target genes in the SCN during light entrainment would provide molecular hints of how the endogenous SCN clock works to achieve its output.

In addition to light, the phase of endogenous circadian rhythms is modulated by the indoleamine neurohormonal transmitters serotonin (5-HT) and melatonin, each of which has a distinct phase response curve. Biochemical studies indicated that the mechanisms by which these indolamines cause phase shifts

was mediated in part by G protein-coupled receptors. Application of 5-HT to hypothalamic slice preparations induced phase advances in spontaneous neuronal activity and the pharmacology of the response was consistent with that of the 5-HT_{1A} receptor, although that receptor had not been detected in the SCN. At the time, no receptor for melatonin had been isolated, but melatonin has a similar chemical structure to 5-HT, hence we thought its receptor might be related to 5-HT receptors. These observations suggested to us that a study of molecules expressed within the SCN that are related to serotonin receptors might lead to identification of proteins involved in the mediation of indoleamine signaling in relation to rhythmicity and also suggested a molecular strategy that would allow their identification. Cloning of the appropriate receptors would be instrumental to identifying the neurons in the SCN region that are the targets of serotonin and melatonin, in designing experiments to determine the mechanism by which these indoleamines entrain the SCN clock (and hence give insights into the nature of the clock itself), and in development of pharmaceutical agents that can alter the clock by interacting with the receptors.

B. New Serotonin Receptors

When we began this study, genes encoding 3 subtypes of 5-HT receptor had been cloned, and these encoded G-protein-associated molecules that span the plasma membrane seven times. To isolate clones of indoleamine-binding receptors from the hypothalamus that belong to the superfamily of G protein-associated receptors, we used a PCR strategy with a rat hypothalamus cDNA template and degenerate primers corresponding to conserved regions of transmembrane domains of the 3 known receptors. Two different variations of this general strategy were used to isolate partial clones of four novel putative receptors. We used the PCR-derived cDNAs as probes to screen a rat hypothalamus cDNA library or rat genomic library to obtain clones spanning the entire protein coding region for each receptor, subsequently named 5-HT_{1F}, 5-HT_{5A}, 5-HT_{5B} and 5-HT_7 (see publications). We determined their nucleotide sequences and found that each putatively encoded a protein with the amino acid sequence characteristic of members of the G protein-coupled, seven transmembrane domain receptor superfamily. A search of the protein and nucleic acid databases with the primary structures of these four putative receptors revealed that 5-HT_{1F} has significant amino acid sequence identity with known 5-HT receptors that are coupled to the inhibition of adenylate cyclase: human 5-HT_{1E} = 55%, dog 5-HT_{1D} = 48% and rat 5-HT_{1B} = 46%. In contrast, 5-HT_{5A}, 5-HT_{5B} and 5-HT_7 have only 30-35% amino acid sequence identity with both catecholamine and 5-HT receptors alike, with none exhibiting dominant similarity. However, 5-HT_{5A} and 5-HT_{5B} have 68% mutual sequence identity. 5-HT_7 was not closely related to any characterized receptors. Which, if any, of these mediated clock entrainment?

To determine the ligand(s) for these four putative receptors empirically, we subcloned their cDNAs into a eukaryotic

expression vector and transiently expressed the encoded protein is CosM6 cells. We found that broken cell preparations from transfected cells containing either 5-HT_{1F}, 5-HT_{5A}, 5-HT_{5B} or 5-HT_7 exhibited saturable binding for [^{125}I]-LSD, a non-selective serotonergic ligand: the calculated equilibrium dissociation constants (K_D) for the individual receptors was 14, 0.64, 2 and 1.5 nM, respectively. We next tested the ability of several biogenic amine neurotransmitters to displace [^{125}I]-LSD binding from these receptors. Serotonin was able to displace [^{125}I]-LSD binding to all receptors; neurohormones melatonin, dopamine and epinephrine had no measurable effects.

C. Anatomical Distributions

We performed Northern blots with RNA extracted from eight brain regions (cortex, hypothalamus, thalamus, hippocampus, striatum, pons, medulla and cerebellum) and heart, liver and kidney. 5-HT_{5B} mRNA was detected only in the hippocampal sample. In situ hybridization studies confirmed the hippocampal expression and additionally detected the mRNA in the medial habenula. 5-HT_{5A} mRNAs were detected in hippocampus > cortex = thalamus = pons = striatum = medulla. Subsequent studies with antibodies made against this receptor have shown that its expression is prominent in astrocytes. 5-HT_7 mRNAs were found in hypothalamus > thalamus > pons = hippocampus. 5-HT_{1F} was not detected in any of the tissues examined by this method, however, by using a semi-quantitative PCR technique we found 5-HT_{1F} mRNAs in cortex = striatum = hippocampus > thalamus = pons > hypothalamus > cerebellum. None of the receptor mRNAs was detected in heart, liver or kidney.

D. Pharmacological and Functional Parameters

To relate each of these receptors to known members of the 5-HT receptor family, we performed three analyses. We determined the ability of selective 5-HT agonists and antagonists to displace LSD from the membrane preparations. We compared their primary structures with other known 5-HT receptors by a dendrogram analysis (during the 1.5 year interval between when we initiated our study until we had complete structures and binding data, a total of 12 mammalian 5-HT receptors, including our 4, became known). We also expressed each receptor in HeLa cells to determine its ability to mediate cAMP accumulation.

[^{125}I]-LSD binding to the 5-HT_{1F} protein was sensitive to sumatriptan, a 5-HT_{1D} agonist, but insensitive to 5-carboxyamido tryptamine (5-CT), a mixed 5-$HT_{1A/1D}$ agonist, and 8-OH-DPAT, a 5-HT_{1A} agonist. The pharmacological profile was similar to, although distinct from, that of the 5-HT_{1E} receptor. Because 5-HT_{1F} had greatest identity (55%) with the recently cloned 5-HT_{1E} receptor, we concluded that we had identified a close relative of the 5-HT_{1E} receptor that is a new member of the 5-HT_1 (5-$HT_{1A/B/D/E}$) family. In functional terms, we showed that 5-HT_{1F} mediates the inhibition of cAMP accumulation in HeLa cells, presumably through

a G_i-protein, as do the other members of this family.

5-HT_{5A} and 5-HT_{5B} had similar pharmacological profiles which did not readily fit with any pharmacologically defined 5-HT receptors that have been previously described. Both receptors were sensitive to ergotamine, 5-CT and methiothepin but insensitive to sumatriptan and 8-OH DPAT. In dendrogram analyses, the primary structures of 5-HT_{5A} and 5-HT_{5B} lie in between those of the 5-HT_1 and 5-HT_2 families. Thus, these receptors form their own subfamily. We were unable to measure linkage of either 5-HT_{5A} or 5-HT_{5B} to a functional response: neither receptor mediated stimulation or inhibition of cAMP accumulation in HeLa cells. They may couple to a second messenger other than cAMP. Recently, we have produced antisera directed against 5-HT_{5A} and found it by immunohistochemistry to be expressed predominantly in astrocytes. Thus, it is possible that the transfected HeLa cells lack a critical component of the receptor-astrocyte coupling pathway. Ongoing studies have been designed to test this notion.

5-HT_7 had a pharmacological profile that was unique. Displacement of [^{125}I]-LSD binding was sensitive to 5-HT_{1A} agonists 8-OH DPAT and 5-CT but also sensitive to the 5-HT_2 antagonist ritanserin. The 5-HT_{1A} antagonist pindolol did not displace LSD. Comparison of the 5-HT_7 primary structure with those of other known 5-HT receptors showed that it was not closely related to any (including the recently cloned 5-HT_6 receptor), thus it inaugurates a new subfamily. Stimulation of 5-HT_7 by serotonin, 5-CT or 8-OH DPAT caused the accumulation of cAMP in HeLa cells. The effect was antagonized by ritanserin and NAN-190 but not by pindolol. Thus, 5-HT_7 couples to a G_s protein. Its responsiveness to 5-CT and 8-OH DPAT and inhibition by ritanserin provide a unique signature among known 5-HT receptors by which it can be identified in physiological studies.

Each of the four new receptors had unique properties. Apart from their different pharmacological profiles (which although this feature may be useful for finding drugs that discriminate receptors, presumably it has at best secondary relevance to the normal activities of the receptors in vivo), each is expressed in a different set of neural cells and each subfamily couples a different second messenger system. Thus, the primary biological significance of the large redundancy of receptors appears to be explained by the desirability of transducing a serotonin signal via distinct mechanisms in different subsets of cells (and perhaps at different subcellular locations).

E. Genetic Mapping

To determine whether any of these receptors corresponded to a locus for which a mouse or human mutant already existed, we assigned each to the mouse genetic map. We used RFLP analysis on DNA extracted from a characterized panel of interspecific backcross mice to assign the Htr1f, Htr5a, Htr5b and Htr7 loci to

chromosomes 16, 5, 1 and 19, respectively. None of these positions corresponded to a candidate mouse mutant, although the Htr5b assignment is in a region of synteny with the human locus for Tourette syndrome, which may involve a deficit in serotonin mechanisms.

F. Experimental Implication of 5-HT_7 In Circadian Rhythm

We examined the mRNA expression patterns, pharmacological profiles and functional properties of each receptor to determine whether these qualities matched previously identified properties of 5-HT receptors implicated in SCN-clock mechanisms. The 5-HT_{1F}, 5-HT_{5A} and 5-HT_{5B} receptors each had one or more property that precluded its involvement. However, 5-HT_7 had features that made it a particularly good candidate for mediating 5-HT-induced phase advances in the SCN. It was enriched in the hypothalamus. Its pharmacology was consistent with the observations that serotonin, 5-CT and 8-OH DPAT induced phase advances in the spontaneous electrical activity of hypothalamic slice explants optimally at CT7 (7 hours after lights on), an effect that was antagonized by NAN-190 and mimicked by cAMP analogues and phosphodiesterase inhibitors. This had previously been interpreted as evidence for a 5-HT_{1A} receptor paradoxically coupled to stimulation of cAMP, but our studies suggested the alternative hypothesis that 5-HT_7 was the active receptor in these assays.

To discriminate between the alternative hypotheses of 5-HT_{1A} or 5-HT_7 involvement, we analyzed the pharmacological profile of phase advance using the hypothalamic slice assay and compared the results to the distinct pharmacological signatures of these two receptors. 8-OH DPAT applied at CT7 elicited a 3 hour phase advance in spontaneous neuronal activity that was antagonized by ritanserin but not pindolol. Ritanserin by itself (without agonist) had no effect. Since 5-HT_{1A} is antagonized by pindolol but not ritanserin, it cannot be the target receptor. Ritanserin is an antagonist for receptors additional to 5-HT_7, but none of these is agonized by 8-OH DPAT. Thus, among known 5-HT receptors, only 5-HT_7 is consistent with the pharmacological profile of the slice. After a frenetic year of discovery in 1992-93, only one additional 5-HT receptor has been identified, suggesting that most if not all have already been found.

G. Isolation Of Human Homolog

The human homolog of 5-HT_7 has been isolated by PCR amplification from a human hypothalamus cDNA library. Progress was initially slow because available human libraries proved to be of low quality. We were unable to isolate clones with large inserts by direct screening or to amplify the entire 5-HT_7 open reading frame in a single step, probably because full-length clones are rarely or not represented in the libraries. The strategy which proved successful involved stepwise independent amplification of 5′ and 3′ fragments which overlapped at an

internal XhoI site, followed by assembly of the cloned fragments into a baculovirus expression vector. During confirmation of the sequence, a one-base frame shift was detected 4 amino acids from the carboxy terminus. This was been repaired by synthesizing appropriate PCR primers. The human clone was inserted into a baculovirus expression vector and its complete sequence verified.

H. Mapping receptor distribution

Immunochemical mapping of the 5-HT_7 protein to SCN neurons would greatly strengthen our hypothesis that it is the receptor that mediates phase shifts. Furthermore, its location on SCN neurons may provide clues as to how it serves to initiate phase shifts by limiting possible mechanisms. The Northern blotting studies mentioned above demonstrated that 5-HT_7 mRNA is predominantly expressed in hypothalamus and thalamus. In situ hybridization studies in the SCN region were inconclusive in that we detected the receptor RNA in some fields the SCN, but more frequently in the subparaventricular zone dorsal to the SCN.

To establish this point unambiguously, we chemically synthesized six peptides corresponding to putative non-membrane-spanning regions of the 5-HT_7 protein sequence and used these to immunize two rabbits each. To demonstrate that the antisera detect the 5-HT_7 receptor protein, we examined extractes from insect cells infected with the bacculovirus construct and showed that that they contained an immunoreactive glycoprotein with 66-70 kDa mobility present in the membrane fraction of infected but not uninfected cells, consistent with proper glycosylation and membrane insertion of the 448 amino acid protein. This study demonstrates the specificity of the antisera in a Western blot format. Several sera had high titer specific immunochemical reactivity that was blocked when the sera were preincubated with the peptide immunogen. The positive sera at dilutions of 1:5000 stained neuronal soma with a predominant distribution within the hypothalamus, especially the ventral region, and nuclei in the dorsal thalamus. The overall distribution of the target cells is consistent with RNA data. Within the SCN region the staining is consistent with a subparaventricular zone distribution, although some further studies are necessary to make this statement with certainty. Preliminary data suggest obtained by PCR suggest that the concentration of the receptor mRNA cycles, with highest concentration at CT15.

I. Model For Role Of 5-HT In Circadian Regulation

The data suggest that the 5-HT_7 receptor is synthesized by subparaventricular zone neurons and cycles in concentration during the day. It is optimally available for binding serotonin at about noon (half a day out of phase with its mRNA). Binding activates G_s, which stimulates adenylyl cyclase to produce cAMP, which in turn activates PKA. The exact compartmental localization of the receptor within the neuron remains to be elucidated, as do the targets of PKA activation and how they

effect a phase shift.

J. Generation of Knock-out mice

To test the guiding hypothesis, we are inactivating the 5-HT_7 gene by homologous targeting in embryo stem cells. We used the rat cDNA clone to isolate the homologous mouse gene from a library constructed in λDashII from genomic DNA isolated from strain 129, the same strain from which the embryonic stem cell line E14 was derived, so as to guarantee complete identity in the recombination sequences. We determined the sequence of 5kb of the 5-HT_7 gene containing its 0.8kb exon II. We inserted the neomycin expression cassette pPol2, which directs neo expression from the RNA polymerase II promoter, into the portion of the 5-HT_7 coding region immediately preceding that encoding the fourth transmembrane domain. We have verified the sequence of this construct, which should direct expression of a nonfunctional, truncated 5-HT_7 protein. A diphtheria toxin expression cassette, derived from pMCIDT, has been attached to the short arm of the knock-out construct so that non-homologous integrants will be selected against. The construct has been introduced into the E14 ES cell line by electroporation and G418 resistant transfectants isolated. These are being assayed for homologous integrants, and positive clones will be used to establish knock-out mouse lines for anatomical, physiological and behavioral studies.

K. Cloning of SCN-cycling Genes

In addition to these focussed studies on one of the receptors that mediates entrainment by serotonin, we have taken a more global molecular approach to elucidating the proteins that constitute the clock entrainment mechanism in the SCN. We have developed new technology to allow us to identify cDNAs corresponding to mRNAs that are specific to or that cycle within the SCN or that are induced by an entraining stimulus. The initial experiments follow from the findings by others (discussed above) that immediate early genes are induced selectively in the SCN in response to light. Because IEGs initiate cell-specific biochemical cascades, such a catalog should provide a great deal of information regarding how the entrainment mechanism works in the SCN. These studies are being carried out in collaboration with Dr. Michael Rea.

Target cDNA libraries have been prepared from RNAs extracted from punched SCNs of entrained hamsters 30 or 180 minutes after a short exposure to light at CT19. Driver cDNA libraries have been prepared from control hamsters not exposed to the light pulse but sacrificed at the same time as the experimental animals. For each sample, double-stranded cDNA was prepared from 10-30 ng of polyA-enriched RNA using a $NotIT_{18}$ primer to initiate cDNA synthesis at the 3′ poly A tract. After sonication to shear the cDNA fragments to an average 500bp and end polishing, they were ligated to short synthetic double-stranded linker fragments containing a site for EcoRI cleavage. Primers corresponding to

the linker sequence, which was present on both ends of the cDNA fragments, were used in the PCR to amplify the mass of cDNA. Following cleavage with EcoRI and NotI, the target and driver cDNAs were directionally cloned into different RNA expression vectors.

The libraries were examined by two techniques. First, PCR primer pairs specific for c-fos mRNA or the ubiquitously expressed mRNA encoding cyclophilin were used on aliquots of the 30 minute target and driver libraries. The cyclophilin primers gave products of the expected size and equal band intensities with both libraries, whereas the fos primers gave a product of the expected size with only the target library. Second, the two target and two driver libraries were amplified and aliquots were digested with the restriction endonuclease HaeIII. The digested library samples were subjected to electrophoresis and blotted to membranes, which were used as targets for Southern blotting. Strong hybridization to a few bands in the 30 minute target library, and much weaker hybridization to the same bands in the 180 minute target library were observed with a probe for c-fos, but no bands were detected in the driver libraries. A comparable result was obtained for another IEG, NGF1a, known to be induced by light entrainment. The set of bands detected by a cyclophilin probe were indistinguishable in the four libraries. These so-called cDNA library Southern blot studies suggest that the entrainment paradigm used to produce the RNA samples worked as expected, and that the cDNA libraries are representative of the mRNA populations in the starting tissue samples.

To identify the repertoire of mRNAs whose expression is elevated at 30 minutes after light exposure and those which either remain elevated at or which are subsequently induced at 180 minutes post-exposure, we performed directional tag PCR subtractive hybridization. Transcripts from the two target libraries were reverse transcribed in the presence of trace-labeled nucleotide triphosphates, and hybridized in two cycles to an excess of RNA transcribed from the respective driver library. After the second hydroxyapatite column, less than 3% of the target remained in the single stranded fraction in each subtraction. The subtracted material was cloned, and the inserts PCR amplified and labeled from the 30 minute subtracted library were compared in a filter hybridization experiment to the inserts prepared in the same manner from the 30 minute unsubtracted target library. Whereas the unsubtracted inserts hybridized very strongly to cyclophilin but at background levels to c-fos, the subtracted library inserts hybridized very strongly to c-fos but at background levels to cyclophilin, consistent with a 30-fold increase in the c-fos specific activity, and presumably comparable increases in other entrainment-activated species. Several hundred clones from the two subtracted libraries have been screened with the subtracted probe. Thus far, over 30 candidate clones have been positive. The sequences of most of these are novel in data base searches. Primer pairs specific for each novel clone have been sent to Dr. Rea for validation by PCR

and in situ hybridization. The validated clones will serve as the main resource for our future studies.

IV. PUBLICATIONS

1. Lovenberg, T.W., M.G. Erlander, B.M. Baron, M. Racke, A.L. Slone, B.W. Siegel, C.M. Craft, J.E. Burns, P.E. Danielson, and J.G. Sutcliffe (1993) Molecular cloning and functional expression of a novel rat and human 5-hydroxytryptamine receptor gene. Proc. Natl. Acad. Sci. USA 90:2184-2188.

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4. Lovenberg, T.W., B.M. Baron, L. de Lecea, J.D. Miller, R.A. Prosser, M.A. Rea, P.E. Foye, M. Racke, A.L. Stone, B.W. Siegel, P.E. Danielson, J.G. Sutcliffe and M.G. Erlander (1993) A novel adenylate cyclase-activating serotonin receptor ($5\text{-}HT_7$) implicated in the regulation of mammalian circadian rhythms. Neuron 11:449-458.

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6. Danielson, P.E., J.B. Watson, D. Gerendasy, M.G. Erlander, T.W. Lovenberg, L. de Lecea, J.G. Sutcliffe and W.N. Frankel (1994) Chromosomal mapping of mouse genes expressed selectively within the central nervous system. Genomics 19:454-461.

V. PERSONNEL

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The Scripps Research Institute

J. Gregor Sutcliffe, Ph.D.
Mark G. Erlander, Ph.D.
Timothy W. Lovenberg, Ph.D.
Guy Barry
Monica Carson, Ph.D.
Pamela E. Foye

Patria Danielson
Jeffrey E. Burns
Ana Dopazo, Ph.D.
Luis deLecea, Ph.D.
Keith Cannon
Janine Dowgialo
Laurie McPherson

Other Collaborators

Bruce M. Baron, Ph.D.
Margaret Racke
Amy L. Slone
Barry W. Siegel
M.P. Cagle
R.L. Gannon
Joseph D. Miller, Ph.D.
Rebecca A. Prosser, Ph.D.
Michael A. Rea, Ph.D.

VI. INTERACTIONS

AFOSR Chonobiology Program, San Antonio, Texas (Sutcliffe, Erlander)
Concepts in Biology and Medicine, Scripps Faculty Lecture Series (Sutcliffe)
Advances in the Pharmacology and Clinical Applications of Serotonin, Kona, Hawaii (Sutcliffe, Erlander, Lovenberg)
Society for Research on Biological Rhythms, Amelia Island (Erlander)
Elba International Neuroscience Program, Marino di Campo, Italy (Sutcliffe)
World Congress on Psychiatric Genetics, New Orleans, (Sutcliffe)
Departments of Anatomy, Cell Biology and Physiology, UCLA (Sutcliffe)
Society for Neuroscience, Washington, DC (Sutcliffe, Erlander, deLecea, Dopazo)
American Society for Neurochemistry, Albuquerque, 1994, (Sutcliffe, Carson)
Northwestern University Medical School, Chicago (Sutcliffe)
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UC Irvine, Dept of Anatomy & Neurobiology, Irvine (Erlander)
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VII. INVENTIONS

"Human Serotonin Receptors, DNA Encoing the Receptors and Uses Thereof" TSR #5099P
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